

WHAT IS CLAIMED IS:

- 1                   1. A system for forming a connective tissue construct, comprising:  
2                   a substrate;  
3                   at least two anchors secured to the substrate in spaced relationship;  
4           and  
5                   fibroblast cells provided on the substrate in the absence of a synthetic  
6           matrix, wherein at least some of the cells are in contact with the anchors, the cells  
7           cultured *in vitro* under conditions to allow the cells to become confluent between the  
8           anchors,  
9                   wherein the anchors are receptive to the cells and allow the cells to  
10           attach thereto while permitting the cells to detach from the substrate to form a three-  
11           dimensional connective tissue construct.
- 1                   2. The system according to claim 1, wherein the fibroblast cells are  
2           derived from tendon tissue.
- 1                   3. The system according to claim 1, wherein the fibroblast cells are  
2           derived from ligament tissue.
- 1                   4. The system according to claim 1, wherein the fibroblast cells are  
2           derived from stem cells.
- 1                   5. The system according to claim 1, wherein the fibroblast cells self-  
2           organize to form the three-dimensional connective tissue construct.
- 1                   6. The system according to claim 1, wherein the anchors include silk  
2           suture segments coated with cell adhesion molecules.
- 1                   7. The system according to claim 6, wherein the cell adhesion  
2           molecules include laminin.

1                   8. The system according to claim 1, wherein the anchors include a  
2 bone-like substrate.

1                   9. The system according to claim 1, wherein the substrate is coated  
2 with cell adhesion molecules.

1                   10. The system according to claim 9, wherein the cell adhesion  
2 molecules include laminin.

1                   11. The system according to claim 10, wherein the concentration of  
2 laminin is about 1.5 to 3.0  $\mu\text{g}/\text{cm}^2$ .

1                   12. The system according to claim 9, wherein the cell adhesion  
2 molecules include thrombin.

1                   13. The system according to claim 1, wherein the substrate and  
2 anchors are incubated with a growth medium prior to providing fibroblast cells on  
3 the substrate.

1                   14. The system according to claim 1, wherein the fibroblast cells are  
2 disposed in a growth medium prior to becoming confluent, and are disposed in a  
3 differentiation medium after becoming confluent.

1                   15. The system according to claim 1, wherein the fibroblast cells are  
2 supplemented with ascorbic acid.

1                   16. The system according to claim 15, wherein the ascorbic acid  
2 includes approximately 100  $\mu\text{g}/\text{ml}$  of L-ascorbic acid 2-phosphate.

1                   17. The system according to claim 1, further comprising myogenic  
2 precursor cells cultured in combination with the fibroblast cells.

1                   18. A system for forming a tendon construct, comprising:  
2                   a substrate;  
3                   at least two anchors secured to the substrate in spaced relationship;  
4    and  
5                   a medium including fibroblast cells and ascorbic acid provided on the  
6    substrate, wherein at least some of the cells are in contact with the anchors,  
7                   wherein the cells are cultured *in vitro* under conditions to allow the  
8    cells to self-organize and become confluent between the anchors, and the anchors  
9    are receptive to the cells and allow the cells to attach thereto while permitting the  
10   cells to detach from the substrate to form a three-dimensional tendon construct.

1                   19. A method for forming a connective tissue construct, comprising:  
2                   providing a substrate;  
3                   securing at least two anchors to the substrate in spaced relationship;  
4                   providing fibroblast cells on the substrate in the absence of a synthetic  
5    matrix, wherein at least some of the cells are in contact with the anchors; and  
6                   culturing the fibroblast cells *in vitro* under conditions to allow the  
7    cells to become confluent between the anchors,  
8                   wherein the anchors are receptive to the cells and allow the cells to  
9    attach thereto while permitting the cells to detach from the substrate and form a  
10   three-dimensional connective tissue construct.

1                   20. The method according to claim 19, wherein providing fibroblast  
2    cells includes deriving the fibroblast cells from tendon tissue.

1                   21. The method according to claim 19, wherein providing fibroblast  
2    cells includes deriving the fibroblast cells from tendon tissue.

1                   22. The method according to claim 19, wherein providing the  
2    fibroblast cells includes deriving the fibroblast cells from stem cells.

1                   23. The method according to claim 19, wherein culturing the  
2 fibroblast cells allows the cells to self-organize to form the three-dimensional  
3 connective tissue construct.

1                   24. The method according to claim 19, wherein the anchors include  
2 silk suture segments coated with cell adhesion molecules.

1                   25. The method according to claim 24, wherein the cell adhesion  
2 molecules include laminin.

1                   26. The method according to claim 19, wherein the anchors include  
2 a bone-like substrate.

1                   27. The method according to claim 19, further comprising coating  
2 the substrate with cell adhesion molecules.

1                   28. The method according to claim 27, wherein the cell adhesion  
2 molecules include laminin.

1                   29. The method according to claim 28, wherein the concentration of  
2 laminin is about 1.5 to 3.0  $\mu\text{g}/\text{cm}^2$ .

1                   30. The method according to claim 27, wherein the cell adhesion  
2 molecules include thrombin.

1                   31. The method according to claim 19, further comprising incubating  
2 the substrate and anchors with a growth medium prior to providing fibroblast cells  
3 on the substrate.

1                   32. The method according to claim 19, further comprising disposing  
2 the fibroblast cells in a growth medium prior to becoming confluent, and disposing  
3 the fibroblast cells in a differentiation medium after becoming confluent.

1                   33. The method according to claim 19, further comprising  
2 supplementing the fibroblast cells with ascorbic acid.

1                   34. The method according to claim 33, wherein the ascorbic acid  
2 includes approximately 100  $\mu\text{g/ml}$  of L-ascorbic acid 2-phosphate.

1                   35. The method according to claim 19, further comprising measuring  
2 a functional property of the connective tissue construct and using the measured  
3 property as feedback to control the formation of the connective tissue construct.

1                   36. The method according to claim 35, wherein the functional  
2 property includes a tensile strength of the connective tissue construct.

1                   37. The method according to claim 19, further comprising culturing  
2 myogenic precursor cells in combination with the fibroblast cells.

1                   38. The method according to claim 19, further comprising harvesting  
2 the fibroblast cells from mammalian tissue.

1                   39. The method according to claim 19, further including implanting  
2 the connective tissue construct in a suitable recipient.

1                   40. A method for forming a tendon construct, comprising:  
2 providing a substrate;  
3 securing at least two anchors to the substrate in spaced relationship;  
4 providing a medium including fibroblast cells and ascorbic acid on  
5 the substrate, wherein at least some of the cells are in contact with the anchors; and  
6 culturing the fibroblast cells *in vitro* under conditions to allow the  
7 cells to self-organize and become confluent between the anchors,  
8 wherein the anchors are receptive to the cells and allow the cells to  
9 attach thereto while permitting the cells to detach from the substrate and form a  
10 three-dimensional tendon construct.